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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Attorney's Docket Number: 06727/010002
U.S. Application Number: PCT/US00/24129

INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/24129	September 1, 2000	September 2, 1999

TITLE OF INVENTION:	USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS
APPLICANTS FOR DO/EO/US:	Robert A. Murgita, Robert Mulroy, and Stace Lindsay

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1.	■ This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.
2.	□ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.
3.	■ This is an express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4.	■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5.	A copy of the International Application as filed (35 U.S.C. § 371(c)(2)). ■ a. is transmitted herewith (required only if not transmitted by the International Bureau). □ b. has been transmitted by the International Bureau. □ c. Is not required, as the application was filed with the United States Receiving Office (RO/US).
6.	□ A translation of the International Application into English (35 U.S.C. § 371(c)(2).
7.	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3)). □ a. are transmitted herewith (required only if not transmitted by the International Bureau). □ b. have been transmitted by the International Bureau. □ c. have not been made; however, the time limit for making such amendments has NOT expired. ⊠ d. have not been made and will not be made.
8.	□ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9.	■ An oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).
10.	□ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371 (c)(5).
11.	□ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12.	□ An assignment for recording. A separate cover sheet in compliance with 37 §§ 3.28 and 3.31 is included.
13.	□ A FIRST preliminary amendment. □ A SECOND or SUBSEQUENT preliminary amendment.
14.	□ A substitute specification.
15.	□ A change of power of attorney and/or address letter.
16.	■ Other items or information: Copy of PCT Application WO 01/15709, Sequence Listing, Diskette, Postcard, Check

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17.	■ The following fees are submitted:		
	BASIC NATIONAL FEE (37 C.F.R. § 1.492(A)(1)-(5)):		
	Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$ 1040.00	
	International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$ 890.00	
	International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO	$740.00	
X	International preliminary examination fee (37 C.F.R. § 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4)	$ 710.00	$710.00
	International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$ 100.00	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$710.00	
Surcharge of $130 for furnishing the oath or declaration later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	[**] - 20 =		x $18	$	
Independent claims	[**] - 3 =		x $84	$	
Multiple dependent claims (if applicable)			+ $280	$	
TOTAL OF ABOVE CALCULATIONS =				$710.00	
Reduction of 1/2 for filing by small entity, if applicable.				$710.00/2 = $355.00	
SUBTOTAL =				$355.00	
Processing fee of $130.00 for furnishing the English translation later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). $40.00 per property. +				$	
TOTAL FEES ENCLOSED =				$	
				Amount to be refunded	$
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■ a. A check in the amount of $355.00 to cover the above fees is enclosed.
□ b. Please charge my Deposit Account No. 03-2095 in the amount of $ [**.**] to cover the above fees.
■ c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 03-2095.

NOTE: Where an appropriate time limit under 37 C.F.R. §§ 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Robert A. Murgita et al.	Art Unit:	Not Yet Assigned
Serial No.:	10/069,623	Examiner:	Not Yet Assigned
Filed:	February 26, 2002	Customer No.:	21559
Title:	USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS		

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows.

In the Specification:

Please insert the following after the title.

--Cross Reference To Related Applications

This application claims priority under 35 U.S.C. § 371 to PCT Application PCT/US00/24129, filed September 1, 2000, which claims priority from United States Provisional Application No. 60/152,166, filed September 2, 1999.--

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 29, 2003

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21559
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USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS

BACKGROUND OF THE INVENTION

The invention related to methods of inhibiting apoptosis.

There are two general ways in which cells die. The most easily recognized way is by necrosis, which is usually caused by an injury that is severe enough to disrupt cellular homeostasis. Typically, the cell's osmotic pressure is disturbed and, consequently, the cell swells and then ruptures. When the cellular contents are spilled into the surrounding tissue space, an inflammatory response often ensues.

The second general way by which cells die is referred to as apoptosis, or programmed cell death. Apoptosis often occurs so rapidly that it is difficult to detect. This may help to explain why the involvement of apoptosis in a wide spectrum of biological processes has only recently been recognized.

The apoptosis pathway has been highly conserved throughout evolution, and plays a critical role in embryonic development, viral pathogenesis, cancer, autoimmune disorders, and neurodegenerative disease. For example, inappropriate apoptosis may cause or contribute to AIDS, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), retinitis pigmentosa and other diseases of the retina, myelodysplastic syndrome (e.g. aplastic anemia), toxin-induced liver disease, including alcoholism, and ischemic injury (e.g. myocardial infarction, stroke, and reperfusion injury). Conversely, the failure of an apoptosis response has been implicated in the development of cancer, particularly follicular lymphoma, p53-mediated carcinomas, and hormone-dependent tumors, in autoimmune

disorders, such as lupus erythematosis and multiple sclerosis, and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

In patients infected with HIV-1, mature $CD4^+$ T lymphocytes respond to stimulation from mitogens or super-antigens by undergoing apoptosis. However, the great majority of these cells are not infected with the virus. Thus, inappropriate antigen-induced apoptosis could be responsible for the destruction of this vital part of the immune system in early stages of HIV infection.

SUMMARY OF THE INVENTION

In general, the invention features the inhibition of apoptosis in a cell, e.g., a cell in a mammal such as a human patient, by contacting the cell with recombinant alpha-fetaprotein ("rHuAFP") or an effective fragment thereof, or with nucleic acid encoding rHuAFP. The invention, in inhibiting apoptosis, can provide therapy for diseases in which inappropriate apoptosis is a feature, including AIDS or HIV infection, neurodegenerative diseases such as ALS, a myelodysplastic syndrome, or an ischemic injury such as occurs in stroke, myocardial infarction, reperfusion injury, or a toxin-induced liver disease. Other features and advantages of the invention will be apparent from the detailed description of the invention, the drawings, and the claims.

BRIEF SUMMARY OF THE DRAWINGS

Fig. 1 is the nucleotide sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of the cDNA encoding human alpha-fetoprotein, and the amino acid sequences (SEQ ID NOs: 3-8) of rHuAFP fragments.

Fig. 2 is the SDS-PAGE analysis of rHuAFP Fragment I (SEQ ID NO: 8) (Lane A, MW marker; Lane B, native human alpha-fetoprotein (AFP); Lane C, unpurified rAFP; Lane D, rAFP Fragment I, and Lane E, AFP (amino acids 1-590 of Fig. 1, SEQ ID NO: 2).

DETAILED DESCRIPTION OF THE INVENTION

Production of Recombinant Human Alpha-fetoprotein

Recombinant AFP can be produced in any standard recombinant protein production system, including prokaryotic cells such as E. coli, and eukaryotic systems such as yeast, mammalian (e.g., CHO cells) and insect cells. Prokaryotic production of rHuAFP is described in Murgita U.S. Patent No. 5,384,250, hereby incorporated by reference.

The methods of the invention can also employ biologically active fragments of rHuAFP. A biologically active fragment of rHuAFP is one that possesses at least one of the following activities: (a) directs a specific interaction with a target cell, e.g., binds to a cell expressing a receptor that is recognized by rHuAFP (e.g., the membrane of a cancer cell such as MCF-7); or (b) halts, reduces, or inhibits apoptosis (e.g., binds to a cell surface receptor and imparts an anti-apoptosis signal). The ability of rHuAFP fragments to bind to a receptor which is recognized by rHuAFP can be tested using any standard binding assay known in the art.

In general, fragments of rHuAFP are produced according to the techniques of polypeptide expression and purification described in U.S. Patent No. 5,384,250. DNA sequences encoding fragments of rHuAFP can be generated by standard techniques and cloned into expression vectors for expression in recombinant cells. Expressed fragments can be isolated by various chromatographic and/or immunological methods known in the art.

Lysis and fractionation of rHuAFP-containing cells prior to affinity chromatography may be performed by standard methods. Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques In Biochemistry and Molecular Biology, Work and Burdon, eds., Elsevier, 1980).

Recombinant HuAFP fragments can be assayed by immunological procedures, such as Western blot, immunoprecipitation analysis of recombinant cell extracts, or immunofluorescence (using, e.g., the methods descrbed in Ausubel et al., *Current Protocols In Molecular Biology*, Greene Publishing Associates and Wiley Interscience (John Wiley & Sons), New York, 1994).

Useful rHuAFP fragments preferably have at least 20 contiguous amino acids, preferably at least 50 contiguous amino acids, more preferably at least 100 contiguous amino acids, and most preferably at least 200 to 400 or more contiguous amino acids in length.

Recombinant HuAFP fragments of interest include, but are not limited to, Domain I (amino acids 1 (Thr) - 197 (Ser), see Fig. 1, SEQ ID NO: 3), Domain II (amino acids 198(Ser) - 389 (Ser), see Fig. 1, SEQ ID NO: 4), Domain III (amino acids 390 (Gln) - 590 (Val), see Fig. 1, SEQ ID NO: 5), Domain I+II (amino acids 1 (Thr) - 389 (Ser), see Fig. 1, SEQ ID NO: 6), Domain II+III (amino acids 198 (Ser) - 590 (Val), see Fig. 1, SEQ ID NO: 7), and rHuAFP Fragment I (amino acids 266 (Met) - 590 (Val), see Fig. 1, SEQ ID NO: 8).

By "inhibiting apoptosis" is meant a decrease in the number of cells which undergo apoptosis relative to an untreated control. Preferably, the decrease is at least 25%, more preferably the decrease is 50%, and most

preferably the decrease is at least one-fold.

Apoptosis Assays

Apoptosis assays are described in the following references. Assays for apoptosis in lymphocytes are disclosed by, for example: Li et al., "Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein", Science 268:429-431, 1995; Gibellini et al., "Tat-expressing Jurkat cells show an increased resistance to different apoptosis stimuli, including acute human immunodeficiency virus-type 1 (HIV-1) infection:, Br. J. Haematol. 89:24-33, 1995; Martin et al., "HIV-1 infection of human $CD4^+$ T cells *in vitro*. Differential induction of apoptosis in these cells." J. Immunol. 152:330-42, 1994; Terai et al., "Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1", J. Clin Invest. 87:1710-5, 1991; Dhein et al., "Autocrine T-cell suicide mediated by APO-1/(Fas/CD95) 11, Nature 373:438-441, 1995; Katsikis et al., "Fas antigent stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals", J. Exp. Med. 1815:2029-2036, 1995; Estendorp et al., "Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120", Nature 375:497, 1995; DeRossi et al., Virology 198:234-44, 1994.

Assays for apoptosis in fibroblasts are disclosed by, for example: Vossbeck et al;, "Direct transforming activity of TGF-beta on rat fibroblasts," Int. J. Cancer 61:92-97, 1995; Goruppi et al., "Dissection of c-myc domains involved in S phase induction of NIH3T3 fibroblasts," Oncogene 9:1537-44, 1994; Fernandez et al., "Differential sensitivity of normal and Ha-ras transformed C3H mouse embryo fibroblasts to tumor necrosis factor: induction of bcl-2, c-myc, and manganese superoxide dismutase in resistant cells," Oncogene 9:2009-17, 1994; Harrington et al., "c-Myc-induced apoptosis in

fibroblasts is inhibited by specific cytokines," EMBO J., 13:3286-3295, 1994; Itoh et al., "A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen," J. Biol. Chem. 268:10932-7, 1993.

Assays for apoptosis in neuronal cells are disclosed by, for example: Melino et al., "Tissue transglutaminase and apoptosis: sense and antisense transfection studies with human neuroblastoma cells," Mol. Cell Biol. 14:6584-6596, 1994; Rosenbaum et al., "evidence for hypoxiainduced, programmed cell death of cultured neurons," Ann. Neurol. 36:864-870, 1994; Sato et al., "Neuronal differentation of PC12 cells as a result of prevention of cell death by bcl-2," J. Neurobiol 25:1227-1234, 1994; Ferrari et al., "N-acetylcysteine D- and L-stereoisimers prevents apoptosis death of neuronal cells," J. Neurosci. 1516:2857-2866, 1995; Talley et al., "Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA," Mol. Cell Biol. 1585:2359-2366, 1995; Talley et al., "Tumor Necrosis Factor Alpha-Induced Apoptosis in Human Neuronal Cells: Protection by the Antioxidant NAcetylcysteine and the Genes bcl-2 and crmA," Mol. Cell. Biol. 15:2359-2366, 1995; and Walkinshaw et al., "Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. Implication for the treatment of Parkinson's disease," J. Clin. Invest. 95:2458-2464, 1995.

Assays for apoptosis in insect cells are disclosed by, for example: Clem et al., "Prevention of apoptosis by a baculovirus gene during infection of insect cells," Science 254:1388-90, 1991; Crook et al., "An apoptosis-inhibiting baculovirus gene with a zince finger-like motif," J. Virol. 67:2168-74, 1993; Rabizadeh et al., "Expression of the baculovirus p35 gene inhibits mammalian neural cell death," J. Neurochem. 61:2318-21, 1993; Birnbaum et al., "an apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a

polypeptide with Cys/His sequence motifs," J. Virol. 68:2521-8, 1994; and Clem et al., "Control of programmed cell death by the baculovirus genes p35 and IAP," Mol. Cell. Biol. 14:5212-5222, 1994.

Gene Therapy

rHuAFP-encoding genes can be used according to the invention in anti-apoptosis gene therapy. In particular, a functional rHuAFP gene may be used to sustain neuronal cells that undergo apoptosis in the course of a neurodegenerative disease; lymphocytes (i.e., T cells and B cells); or cells that have been injured by ischemia.

Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in apoptosis (for example, epithelial cells) may be used as a gene transfer delivery system for a therapeutic rHuAFP gene construct. Numerous vectors useful for this purpose are known (Miller, Human Gene Therapy 15-15, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis and Anderson, BioTechniques 6:608-614, 1988; Tolstoshev and Anderson, Current Opinion in Biotechnology1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechniques 7:980-990, 1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Patent NO. 5,399,346). Non-viral approaches may also be employed for the introduction of therapeutic DNA into cells otherwise predicted to undergo apoptosis. For example rHuAFP may be introduced into a neuron or a

T cell by lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neurosci. Lett. 117:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Meth. Enz. 101:512, 1983), asialorosonucoid-polylysine conjugation (Wu et al., J. Biol. Chem. 263:14621, 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less preferably, microinjection under surgical conditions (Wolff et al., Science 247:1465, 1990).

For any of the methods described above, the therapeutic rHuAFP DNA construct is preferably applied to the site of the predicted apoptosis event (for example, by injection), or to tissue in the vicinity of the predicted apoptosis event, or to a blood vessel supplying the cells predicted to undergo apoptosis.

rHuAFP expression can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers that preferentially direct gene expression in neural cells, T cells, or B cells may be used to direct rHuAFP expression. Alternatively, if an rHuAFP genomic clone is used in a therapeutic construct, regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Alternatively, rHuAFP gene therapy is accomplished by direct adminstration of the rHuAFP mRNA or antisense rHuAFP mRNA to a cell that is expected to undergo apoptosis. The mRNA may be produced and isolated by any standard technique, but is most readily produced by *in vitro* transcription using an rHuAFP cDNA under the control of a high efficiency promoter (e.g., the T7 promoter). Administration of rHuAFP mRNA to cells

can be carried out by any of the methods for direct nucleic acid adminstration described below.

Ideally, the production of rAFP protein by any gene therapy approach will result in cellular levels of rAFP that are at least equivalent to the normal, cellular level of rHuAFP in an unaffected cell. Treatment by any rHuAFP-mediated gene therapy approach may be combined with more traditional therapies.

Administration of rAFP Polypeptides

Another therapeutic approach of the invention involves adminstration of recombinant rHuAFP, either directly to the site of a predicted apoptosis event (for example, by injection) or systemically (for example, by any conventional recombinant protein adminstration technique). The dosage of rHuAFP depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg are administered per day to an adult in a pharmaceutically-acceptable formulation. Administration may begin before or after the patient is symptomatic. Any appropriate route of adminstration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powers, nasals drops, or aerosols.

Methods well known in the art of making formulations are found, for example, in *Remington's Pharmaceutical Sciences*, (18th edition), ed. A.

Gennaro, 1990, Mack Publishing Company, Easton, PA. Formulations for parenteral adminstration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of rHuAFP.

Treatment with an rHuAFP protein or gene may be combined with more traditional therapies for the disease such as surgery, steroid therapy, or chemotherapy for autoimmune disease; antiviral therapy for AIDS; and tissue plasminogen activator (TPA) for ischemic injury.

Other Embodiments

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the appended claims.

What is claimed is:

1. A method of inhibiting apoptosis in a cell, said method comprising administering to said cell an apoptosis inhibiting amount of rHuAFP or an apoptosis-inhibiting fragment thereof.

2. The method of claim 1, wherein said cell is in a mammal.

3. The method of claim 2, wherein said mammal is human.

4. The method of claim 3, wherein said human is infected with HIV, or has a neurodegenerative disease, a myelodysplastic syndrome, or an ischemic injury.

5. The method of claim 4, wherein said ischemic injury is caused by a myocardial infarction, a stroke, a reperfusion injury, or a toxin-induced liver disease.

6. A method of inhibiting apoptosis in a cell, said method comprising transfecting said cell with nucleic acid encoding rHuAFP or an apoptosis-inhibiting fragment thereof.

7. The method of claim 6, wherein said cell is in a human patient.

8. The method of claim 7, wherein said human patient is infected with HIV, or has a neurodegenerative disease, a myelodyplastic syndrome, or an ischemic injury.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(74) Agent: **CLARK, Paul, T.**; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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WO 01/15709 A1

(54) Title: USE OF rAFP TO INHIBIT OR PREVENT APOPTOSIS

(57) Abstract: A method of inhibiting apoptosis in a cell by administering to the cell an apoptosis inhibiting amount of recombinant human alpha-feta protein or an apoptosis-inhibiting fragment thereof.

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61                          70                                      80                                      90
asp glu gln ser ser gly cys leu glu asn gln leu pro ala phe leu glu glu leu cys his glu lys glu ile leu glu lys tyr gly
GAT GAA GAG TCT TCA GGG TGT TTA GAA AAC CAG CTA CCT GCC TTT CTG GAA GAA CTT TGC CAT GAG AAA GAA ATT TTG GAG AAG TAC GGA (371)

91                          100                                     110                                     120
his ser asp cys cys ser gln ser glu glu gly arg his asn cys phe leu ala his lys lys pro thr pro ala ser ile pro leu phe
CAT TCA GAC TGC TGC AGC CAA AGT GAA GAG GGA AGA CAT AAC TGT TTT CTT GCA CAC AAA AAG CCC ACT CCA GCA TCG ATC CCA CTT TTC (461)

121                         130                                     140                                     150
gln val pro glu pro val thr ser cys glu ala tyr glu glu asp arg glu thr phe met asn lys phe ile tyr glu ile ala arg arg
CAA GTT CCA GAA CCT GTC ACA AGC TGT GAA GCA TAT GAA GAA GAC AGG GAG ACA TTC ATG AAC AAA TTC ATT TAT GAG ATA GCA AGA AGG (551)

151                         160                                     170                                     180
his pro phe leu tyr ala pro thr ile leu leu trp ala ala arg tyr asp lys ile ile pro ser cys cys lys ala glu asn ala val
CAT CCC TTC CTG TAT GCA CCT ACA ATT CTT CTT TGG GCT GCT CGC TAT GAC AAA ATA ATT CCA TCT TGC TGC AAA GCT GAA AAT GCA GTT (641)

181                         190                                     200                                     210
glu cys phe gln thr lys ala ala thr val thr lys glu leu arg glu ser ser leu leu asn gln his ala cys ala val met lys asn
GAA TGC TTC CAA ACA AAG GCA GCA ACA GTT ACA AAA GAA TTA AGA GAA AGC AGC TTG TTA AAT CAA CAT GCA TGT GCA GTA ATG AAA AAT (731)
```

Fig. 1

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211                                     220                                         230                                        240
phe gly thr arg thr phe gln ala ile thr val thr lys leu ser gln lys phe thr lys val asn phe thr glu ile gln lys leu val
TTT GGG ACC CGA ACT TTC CAA GCC ATA ACT GTT ACT AAA CTG AGT CAG AAG TTT ACC AAA GTT AAT TTT ACT GAA ATC CAG AAA CTA GTC (821)

241                                     250                                         260                                        270
leu asp val ala his val his glu his cys cys arg gly asp val leu asp cys leu gln asp gly glu lys ile met ser tyr ise cys
CTG GAT GTG GCC CAT GTA CAT GAG CAC TGT TGC AGA GGA GAT GTG CTG GAT TGT CTG CAG GAT GGG GAA AAA ATC ATG TCC TAC ATA TGT (911)

271                                     280                                         290                                        300
ser gln gln asp thr leu ser asn lys ile thr glu cys cys lys leu thr thr leu glu arg gly gln cys ile ile his ala glu asn
TCT CAA CAA GAC ACT CTG TCA AAC AAA ATA ACA GAA TGC TGC AAA CTG ACC ACG CTG GAA CGT GGT CAA TGT ATA ATT CAT GCA GAA AAT(1001)

301                                     310                                         320                                        330
asp glu lys pro glu gly leu ser pro asn leu asn arg phe leu gly asp arg asp phe asn gln phe ser ser gly glu lys asn ile
GAT GAA AAA CCT GAA GGT CTA TCT CCA AAT CTA AAC AGG TTT TTA GGA GAT AGA GAT TTT AAC CAA TTT TCT TCA GGG GAA AAA AAT ATC(1091)

331                                     340                                         350                                        360
phe leu ala ser phe val his glu tyr ser arg arg his pro gln leu ala val ser val ile leu arg val ala lys gly tyr gln glu
TTC TTG GCA AGT TTT GTT CAT GAA TAT TCA AGA AGA CAT CCT CAG CTT GCT GTC TCA GTA ATT CTA AGA GTT GCT AAA GGA TAC CAG GAG(1181)

361                                     370                                         380                                        390
leu leu glu lys cys phe gln thr glu asn pro leu glu cys gln asp lys gly glu glu glu leu gln lys tyr ile gln glu ser gln
TTA TTG GAG AAG TGT TTC CAG ACT GAA AAC CCT CTT GAA TGC CAA GAT AAA GGA GAA GAA GAA TTA CAG AAA TAC ATC CAG GAG AGC CAA(1271)

391                                     400                                         410                                        420
ala leu ala lys arg ser cys gly leu phe gln lys leu gly glu tyr tyr leu gln asn ala phe leu val ala tyr thr lys lys ala
GCA TTG GCA AAG CGA AGC TGC GGC CTC TTC CAG AAA CTA GGA GAA TAT TAC TTA CAA AAT GCG TTT CTC GTT GCT TAC ACA AAG AAA GCC(1361)

421                                     430                                         440                                        450
pro gln leu thr ser ser glu leu met ala ile thr arg lys met ala ala thr ala ala thr cys cys gln leu ser glu asp lys leu
CCC CAG CTG ACC TCG TCG GAG CTG ATG GCC ATC ACC AGA AAA ATG GCA GCC ACA GCA GCC ACT TGT TGC CAA CTC AGT GAG GAC AAA CTA(1451)
```

Fig. 1 (CONTINUED)

```
451                                     460                                               470                                               480
leu ala cys gly glu gly ala ala asp ile ile ile gly his leu cys ile arg his glu met thr pro val asn pro gly val gly gln
TTG GCC TGT GGC GAG GGA GCG GCT GAC ATT ATT ATC GGA CAC TTA TGT ATC AGA CAT GAA ATG ACT CCA GTA AAC CCT GGT GTT GGC CAG(1541)

481                                     490                                               500                                               510
cys cys thr ser ser tyr ala asn arg arg pro cys phe ser ser leu val val asp glu thr tyr val pro pro ala phe ser asp asp
TGC TGC ACT TCT TCA TAT GCC AAC AGG AGG CCA TGC TTC AGC AGC TTG GTG GTG GAT GAA ACA TAT GTC CCT CCT GCA TTC TCT GAT GAC(1631)

511                                     520                                               530                                               540
lys phe ile phe his lys asp leu cys gln ala gln gly val ala leu gln thr met lys gln glu phe leu ile asn leu val lys gln
AAG TTC ATT TTC CAT AAG GAT CTG TGC CAA GCT CAG GGT GTA GCG CTG CAA ACG ATG AAG CAA GAG TTT CTC ATT AAC CTT GTG AAG CAA(1721)

541                                     550                                               560                                               570
lys pro gln ile thr glu glu gln leu glu ala val ile ala asp phe ser gly leu leu glu lys cys cys gln gly gln glu gln glu
AAG CCA CAA ATA ACA GAG GAA CAA CTT GAG GCT GTC ATT GCA GAT TTC TCA GGC CTG TTG GAG AAA TGC TGC CAA GGC CAG GAA CAG GAA(1811)

571                                     580                                               590
val cys phe ala glu glu gly gln lys leu ile ser lys thr arg ala ala leu gly val ter
GTC TGC TTT GCT GAA GAG GGA CAA AAA CTG ATT TCA AAA ACT CGT GCT GCT TTG GGA GTT TAA  ATTACTTCAGGGGAAGAGAAGACAAAACGAGTCT(1908)

TTCATTCGGTGTGAACTTTTCTCTTTAATTTTAACTGATTTAACACTTTTTGTGAATTAATGAAATGATAAAGACTTTTATGTGAGATTTCCTTATCACAGAAATAAAATATCTCCAAA(2027)
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Fig. 1 (CONTINUED)

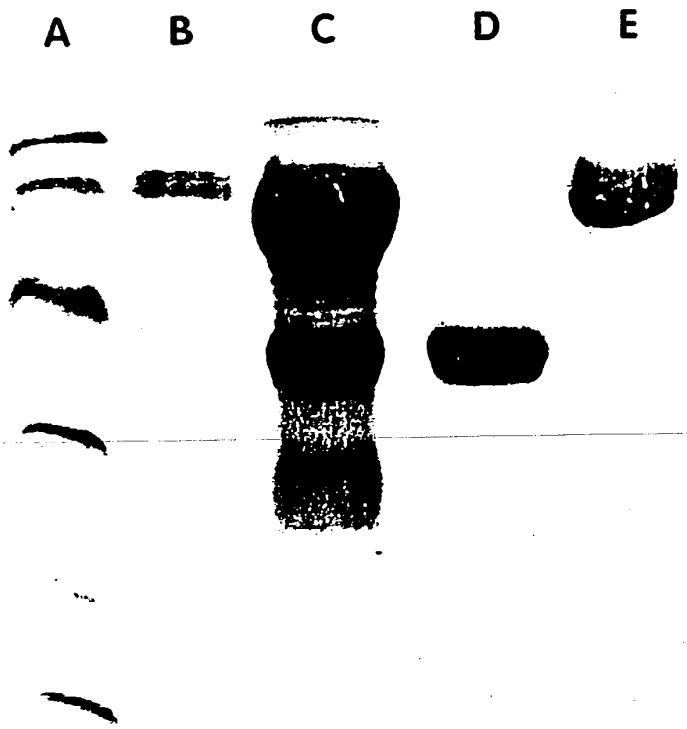

Fig. 2

PATENT
ATTORNEY DOCKET NO: 06727/010002

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled USE OF RAFP TO INHIBIT OR PREVENT APOPTOSIS, the specification of which

~ is attached hereto.
: was filed on February 26, 2002 as Application Serial No. 10/069,623 and was amended on _____.
~ was described and claimed in PCT International Application No. filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, ∋ 1.56.

FOREIGN PRIORITY RIGHTS: I hereby claim foreign priority benefits under Title 35, United States Code, ∋ 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
PCT	PCT/US00/24129	September 1, 1999	Yes/No

PROVISIONAL PRIORITY RIGHTS: I hereby claim priority benefits under Title 35, United States Code, ∋ 119(e) and ∋ 120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status
60/152,166	September 2, 1999	Pending

NON-PROVISIONAL PRIORITY RIGHTS: I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status

I hereby appoint the following attorney(s) and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Krishna Bleker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Vicki Healy, Reg. No. 48,343.

Address all telephone calls to: Paul T. Clark at 617/426-0200.

Address all correspondence to: Paul T. Clark at Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110. **Customer No: 21559**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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Signature: R. A. Murgita			Dec. 10, 2002 Date:

100

COMBINED DECLARATION AND POWER OF ATTORNEY

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Signature:			Date: 11/12/02	

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Signature:			Date:	

COMBINED DECLARATION AND POWER OF ATTORNEY

	Massachusetts USA	Cambridge, Massachusetts 02138 USA	
Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
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Signature: Stace Lindsay			Date: 11/21/02

300

SEQUENCE LISTING

<110> Atlantic Biopharmaceuticals, Inc.
Murgita, Robert A.
Mulroy, Robert
Lindsay, Stace

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            20                  25                  30
Ala Gln Phe Val Gln Glu Ala Thr Tyr Lys Glu Val Ser Lys Met Val
        35                  40                  45
Lys Asp Ala Leu Thr Ala Ile Glu Lys Pro Thr Gly Asp Glu Gln Ser
    50                  55                  60
Ser Gly Cys Leu Glu Asn Gln Leu Pro Ala Phe Leu Glu Glu Leu Cys
65                  70                  75                  80
His Glu Lys Glu Ile Leu Glu Lys Tyr Gly His Ser Asp Cys Cys Ser
                85                  90                  95
Gln Ser Glu Glu Gly Arg His Asn Cys Phe Leu Ala His Lys Lys Pro
            100                 105                 110
Thr Ala Ala Trp Ile Pro Leu Phe Gln Val Pro Glu Pro Val Thr Ser
        115                 120                 125
Cys Glu Ala Tyr Glu Glu Asp Arg Glu Thr Phe Met Asn Lys Phe Ile
    130                 135                 140
Tyr Glu Ile Ala Arg Arg His Pro Phe Leu Tyr Ala Pro Thr Ile Leu
145                 150                 155                 160
Leu Ser Ala Ala Gly Tyr Glu Lys Ile Ile Pro Ser Cys Cys Lys Ala
                165                 170                 175
Glu Asn Ala Val Glu Cys Phe Gln Thr Lys Ala Ala Thr Val Thr Lys
            180                 185                 190
Glu Leu Arg Glu Ser Ser Leu Leu Asn Gln His Ala Cys Pro Val Met
        195                 200                 205
Lys Asn Phe Gly Thr Arg Thr Phe Gln Ala Ile Thr Val Thr Lys Leu
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225                 230                 235                 240
Leu Asp Val Ala His Val His Glu His Cys Cys Arg Ala Asp Val Leu
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Asp Cys Leu Gln Asp Gly Glu Lys Ile Met Ser Tyr Ile Cys Ser Gln
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Gln Asp Thr Leu Ser Asn Lys Ile Thr Glu Cys Cys Lys Leu Thr Thr
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Leu Glu Arg Gly Gln Cys Ile Ile His Ala Glu Asn Asp Glu Lys Pro
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Asn Gln Phe Ser Ser Gly Glu Lys Asn Ile Phe Leu Ala Ser Phe Val
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Glu Asn Pro Leu Glu Cys Gln Asp Lys Gly Glu Glu Glu Leu Gln Lys
    370                 375                 380
Tyr Ile Gln Glu Ser Gln Ala Leu Ala Lys Arg Ser Cys Gly Leu Phe
385                 390                 395                 400
Gln Lys Leu Gly Glu Tyr Tyr Leu Gln Asn Glu Phe Leu Val Ala Tyr
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Thr Lys Lys Ala Pro Gln Leu Thr Ser Ser Glu Leu Met Ala Ile Thr
            420                 425                 430
Arg Lys Met Ala Ala Thr Ala Ala Thr Cys Cys Gln Leu Ser Glu Asp
        435                 440                 445
Lys Leu Leu Ala Cys Gly Glu Gly Ala Ala Asp Ile Ile Ile Gly His
    450                 455                 460
Leu Cys Ile Arg His Glu Met Thr Pro Val Asn Pro Gly Val Gly Gln
465                 470                 475                 480
Cys Cys Thr Ser Ser Tyr Ala Asn Arg Arg Pro Cys Phe Ser Ser Leu
                485                 490                 495
Val Val Asp Glu Thr Tyr Val Pro Pro Ala Phe Ser Asp Asp Lys Phe
            500                 505                 510
Ile Phe His Lys Asp Leu Cys Gln Ala Gln Gly Val Ala Leu Gln Arg
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Met Lys Gln Glu Phe Leu Ile Asn Leu Val Lys Gln Lys Pro Gln Ile
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Thr Glu Glu Gln Leu Glu Ala Leu Ile Ala Asp Phe Ser Gly Leu Leu
545                 550                 555                 560
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        35                  40                  45
Lys Asp Ala Leu Thr Ala Ile Glu Lys Pro Thr Gly Asp Glu Gln Ser
    50                  55                  60
Ser Gly Cys Leu Glu Asn Gln Leu Pro Ala Phe Leu Glu Glu Leu Cys
65                  70                  75                  80
His Glu Lys Glu Ile Leu Glu Lys Tyr Gly His Ser Asp Cys Cys Ser
                85                  90                  95
Gln Ser Glu Glu Gly Arg His Asn Cys Phe Leu Ala His Lys Lys Pro
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            100                 105                 110
Thr Ala Ala Trp Ile Pro Leu Phe Gln Val Pro Glu Pro Val Thr Ser
        115                 120                 125
Cys Glu Ala Tyr Glu Glu Asp Arg Glu Thr Phe Met Asn Lys Phe Ile
    130                 135                 140

Tyr Glu Ile Ala Arg Arg His Pro Phe Leu Tyr Ala Pro Thr Ile Leu
145                 150                 155                 160
Leu Ser Ala Ala Gly Tyr Glu Lys Ile Ile Pro Ser Cys Cys Lys Ala
                165                 170                 175
Glu Asn Ala Val Glu Cys Phe Gln Thr Lys Ala Ala Thr Val Thr Lys
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Glu Leu Arg Glu Ser
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        35                  40                  45
Val His Glu His Cys Cys Arg Ala Asp Val Leu Asp Cys Leu Gln Asp
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Gly Glu Lys Ile Met Ser Tyr Ile Cys Ser Gln Gln Asp Thr Leu Ser
65                  70                  75                  80
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Cys Ile Ile His Ala Glu Asn Asp Glu Lys Pro Glu Gly Leu Ser Pro
            100                 105                 110
Asn Leu Asn Arg Phe Leu Gly Asp Arg Asp Phe Asn Gln Phe Ser Ser
        115                 120                 125
Gly Glu Lys Asn Ile Phe Leu Ala Ser Phe Val His Glu Tyr Ser Arg
    130                 135                 140
Arg His Pro Gln Leu Ala Val Ser Val Ile Leu Arg Val Ala Lys Gly
145                 150                 155                 160
Tyr Gln Glu Leu Leu Glu Lys Cys Phe Gln Thr Glu Asn Pro Leu Glu
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            180                 185                 190
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Gln Leu Thr Ser Ser Glu Leu Met Ala Ile Thr Arg Lys Met Ala Ala
        35                  40                  45
Thr Ala Ala Thr Cys Cys Gln Leu Ser Glu Asp Lys Leu Leu Ala Cys
    50                  55                  60
Gly Glu Gly Ala Ala Asp Ile Ile Ile Gly His Leu Cys Ile Arg His
65                  70                  75                  80
Glu Met Thr Pro Val Asn Pro Gly Val Gly Gln Cys Cys Thr Ser Ser
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Tyr Ala Asn Arg Arg Pro Cys Phe Ser Ser Leu Val Val Asp Glu Thr
            100                 105                 110
Tyr Val Pro Pro Ala Phe Ser Asp Asp Lys Phe Ile Phe His Lys Asp
        115                 120                 125
Leu Cys Gln Ala Gln Gly Val Ala Leu Gln Arg Met Lys Gln Glu Phe
    130                 135                 140
Leu Ile Asn Leu Val Lys Gln Lys Pro Gln Ile Thr Glu Glu Gln Leu
145                 150                 155                 160
Glu Ala Leu Ile Ala Asp Phe Ser Gly Leu Leu Glu Lys Cys Cys Gln
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Lys Asp Ala Leu Thr Ala Ile Glu Lys Pro Thr Gly Asp Glu Gln Ser
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Ser Gly Cys Leu Glu Asn Gln Leu Pro Ala Phe Leu Glu Glu Leu Cys
65                  70                  75                  80
His Glu Lys Glu Ile Leu Glu Lys Tyr Gly His Ser Asp Cys Cys Ser
                85                  90                  95
Gln Ser Glu Glu Gly Arg His Asn Cys Phe Leu Ala His Lys Lys Pro
            100                 105                 110
Thr Ala Ala Trp Ile Pro Leu Phe Gln Val Pro Glu Pro Val Thr Ser
        115                 120                 125
Cys Glu Ala Tyr Glu Glu Asp Arg Glu Thr Phe Met Asn Lys Phe Ile
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Tyr Glu Ile Ala Arg Arg His Pro Phe Leu Tyr Ala Pro Thr Ile Leu
145                 150                 155                 160
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Leu Glu Arg Gly Gln Cys Ile Ile His Ala Glu Asn Asp Glu Lys Pro
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Glu Gly Leu Ser Pro Asn Leu Asn Arg Phe Leu Gly Asp Arg Asp Phe
305                 310                 315                 320
Asn Gln Phe Ser Ser Gly Glu Lys Asn Ile Phe Leu Ala Ser Phe Val
                325                 330                 335
His Glu Tyr Ser Arg Arg His Pro Gln Leu Ala Val Ser Val Ile Leu
            340                 345                 350
Arg Val Ala Lys Gly Tyr Gln Glu Leu Leu Glu Lys Cys Phe Gln Thr
        355                 360                 365
Glu Asn Pro Leu Glu Cys Gln Asp Lys Gly Glu Glu Glu Leu Gln Lys
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Tyr Ile Gln Glu Ser
385
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<210> 7
<211> 393
<212> PRT
<213> Homo sapiens

<400> 7

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Arg Thr Phe Gln Ala Ile Thr Val Thr Lys Leu Ser Gln Lys Phe Thr
            20                  25                  30
Lys Val Asn Phe Thr Glu Ile Gln Lys Leu Val Leu Asp Val Ala His
        35                  40                  45
Val His Glu His Cys Cys Arg Ala Asp Val Leu Asp Cys Leu Gln Asp
    50                  55                  60
Gly Glu Lys Ile Met Ser Tyr Ile Cys Ser Gln Gln Asp Thr Leu Ser
65                  70                  75                  80
Asn Lys Ile Thr Glu Cys Cys Lys Leu Thr Thr Leu Glu Arg Gly Gln
                85                  90                  95
Cys Ile Ile His Ala Glu Asn Asp Glu Lys Pro Glu Gly Leu Ser Pro
            100                 105                 110
Asn Leu Asn Arg Phe Leu Gly Asp Arg Asp Phe Asn Gln Phe Ser Ser
        115                 120                 125
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Gly Glu Lys Asn Ile Phe Leu Ala Ser Phe Val His Glu Tyr Ser Arg
    130                 135                 140
Arg His Pro Gln Leu Ala Val Ser Val Ile Leu Arg Val Ala Lys Gly
145                 150                 155                 160
Tyr Gln Glu Leu Leu Glu Lys Cys Phe Gln Thr Glu Asn Pro Leu Glu
                165                 170                 175
Cys Gln Asp Lys Gly Glu Glu Glu Leu Gln Lys Tyr Ile Gln Glu Ser
            180                 185                 190
Gln Ala Leu Ala Lys Arg Ser Cys Gly Leu Phe Gln Lys Leu Gly Glu
        195                 200                 205
Tyr Tyr Leu Gln Asn Glu Phe Leu Val Ala Tyr Thr Lys Lys Ala Pro
    210                 215                 220
Gln Leu Thr Ser Ser Glu Leu Met Ala Ile Thr Arg Lys Met Ala Ala
225                 230                 235                 240
Thr Ala Ala Thr Cys Cys Gln Leu Ser Glu Asp Lys Leu Leu Ala Cys
                245                 250                 255
Gly Glu Gly Ala Ala Asp Ile Ile Ile Gly His Leu Cys Ile Arg His
            260                 265                 270
Glu Met Thr Pro Val Asn Pro Gly Val Gly Gln Cys Cys Thr Ser Ser
        275                 280                 285
Tyr Ala Asn Arg Arg Pro Cys Phe Ser Ser Leu Val Val Asp Glu Thr
    290                 295                 300
Tyr Val Pro Pro Ala Phe Ser Asp Asp Lys Phe Ile Phe His Lys Asp
305                 310                 315                 320
Leu Cys Gln Ala Gln Gly Val Ala Leu Gln Arg Met Lys Gln Glu Phe
                325                 330                 335
Leu Ile Asn Leu Val Lys Gln Lys Pro Gln Ile Thr Glu Glu Gln Leu
            340                 345                 350
Glu Ala Leu Ile Ala Asp Phe Ser Gly Leu Leu Glu Lys Cys Cys Gln
        355                 360                 365
Gly Gln Glu Gln Glu Val Cys Phe Ala Glu Glu Gly Gln Lys Leu Ile
    370                 375                 380
Ser Lys Thr Gly Ala Ala Leu Gly Val
385                 390
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<210> 8
<211> 325
<212> PRT
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 1                5                  10                  15
Glu Cys Cys Lys Leu Thr Thr Leu Glu Arg Gly Gln Cys Ile Ile His
            20                  25                  30
Ala Glu Asn Asp Glu Lys Pro Glu Gly Leu Ser Pro Asn Leu Asn Arg
        35                  40                  45
Phe Leu Gly Asp Arg Asp Phe Asn Gln Phe Ser Ser Gly Glu Lys Asn
    50                  55                  60
Ile Phe Leu Ala Ser Phe Val His Glu Tyr Ser Arg Arg His Pro Gln
65                  70                  75                  80
Leu Ala Val Ser Val Ile Leu Arg Val Ala Lys Gly Tyr Gln Glu Leu
                85                  90                  95
```

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Leu Glu Lys Cys Phe Gln Thr Glu Asn Pro Leu Glu Cys Gln Asp Lys
            100                 105                 110
Gly Glu Glu Glu Leu Gln Lys Tyr Ile Gln Glu Ser Gln Ala Leu Ala
        115                 120                 125
Lys Arg Ser Cys Gly Leu Phe Gln Lys Leu Gly Glu Tyr Tyr Leu Gln
    130                 135                 140
Asn Glu Phe Leu Val Ala Tyr Thr Lys Lys Ala Pro Gln Leu Thr Ser
145                 150                 155                 160
Ser Glu Leu Met Ala Ile Thr Arg Lys Met Ala Ala Thr Ala Ala Thr
                165                 170                 175
Cys Cys Gln Leu Ser Glu Asp Lys Leu Leu Ala Cys Gly Glu Gly Ala
            180                 185                 190
Ala Asp Ile Ile Ile Gly His Leu Cys Ile Arg His Glu Met Thr Pro
        195                 200                 205
Val Asn Pro Gly Val Gly Gln Cys Cys Thr Ser Ser Tyr Ala Asn Arg
    210                 215                 220
Arg Pro Cys Phe Ser Ser Leu Val Val Asp Glu Thr Tyr Val Pro Pro
225                 230                 235                 240
Ala Phe Ser Asp Asp Lys Phe Ile Phe His Lys Asp Leu Cys Gln Ala
                245                 250                 255
Gln Gly Val Ala Leu Gln Arg Met Lys Gln Glu Phe Leu Ile Asn Leu
            260                 265                 270
Val Lys Gln Lys Pro Gln Ile Thr Glu Glu Gln Leu Glu Ala Leu Ile
        275                 280                 285
Ala Asp Phe Ser Gly Leu Leu Glu Lys Cys Cys Gln Gly Gln Glu Gln
    290                 295                 300
Glu Val Cys Phe Ala Glu Glu Gly Gln Lys Leu Ile Ser Lys Thr Gly
305                 310                 315                 320
Ala Ala Leu Gly Val
                325
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